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ON THE GAMETOPHYTES AND EMBRYO OF TAXODIUM.[1]

CONTRIBUTIONS FROM THE BOTANICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY, No. 1.

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(WITH PLATES I–XI)

IN spite of the recent great increase in our knowledge of spermatogenesis in many groups of gymnosperms, this part of the life history of the Taxodieae remained, at the time this work was undertaken, almost unknown. A short contribution by Shaw ('97) on Sequoia had appeared in 1897, and Arnoldi ('99[a, b]) has recently added two papers on the development of the reproductive organs in Sequoia. These observers have cleared up many salient points in the development of this genus, but the group as a whole is still to be studied.

Taxodium itself, probably on account of its limited geographical distribution, has been greatly neglected by investigators. Coulter on the histology of the leaf, Masters on the seedling, Lotsy and Meehan on the knees, and Von Schrenk on the disease called "peckiness" are among the few papers that have been devoted, in whole or in part, to the study of Taxodium, and none is concerned with the development of the seed.

The present work was suggested by Dr. D. S. Johnson, to whom I wish to express my gratitude for his unfailing kindness and helpful advice throughout its prosecution. I also wish

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publicly to thank my brothers for their assistance in sending me material at frequent intervals.

METHODS.

Collections of *Taxodium distichum* Richard, the only species studied, have been made for about three years, chiefly from Hartsville, S. C., but also from Baltimore, Md., and New Berne, N. C. Fixing has been done at the tree in all critical stages, but fresh material, sent in tight boxes from Hartsville to Baltimore, has frequently given good results. Flemming's strong solution, chrom-acetic acid solution, alcoholic solution of picric acid, saturated solution of corrosive sublimate in 95 per cent. alcohol have all been used to some extent; but a saturated aqueous solution of corrosive sublimate (95 or 90 parts) and glacial acetic acid (5 or 10 parts) has been generally used. The latter gives results that are scarcely, if at all, inferior to those obtained with the Flemming solution, while it is more satisfactory than any of the other fluids mentioned. In searching for protoplasmic connections between cells, Gardner's ('83) methods were used, but only with fixed material. Potassium iodid and chlor-zinc-iodid were useful in determining the presence of starch, and have been used throughout for this purpose. A number of stains have been tried, but Flemming's triple has been most used. Young cones were split, or the scales removed entire. In older cones the ovule was removed and the nucellus exposed by breaking off the lignified tip of the integument, or the whole prothallium was taken from the seed. Sections 5–10 μ thick were made by the usual paraffin method.

THE STAMINATE CONE.

The staminate flowers are born on short branches which are either simple or compound. If simple, these branches are usually longer and more numerous than if compound. They appear in the fall from near the tips of the branches of the same year, and at the beginning of October or even earlier the young staminate flowers may be seen in the axils of their scale-like leaves. A longitudinal section of a sporophyll at this time shows no distinction between primary archesporium and other

tissue, all the cells of the lower part of the sporophyll being of about the same size, and having dense contents. Soon, however, certain centrally placed hypodermal cells begin to divide by periclinal walls and give rise to rows of cells as shown in *fig.* 1. The outermost cells of these rows, by a periclinal division, form the one-layered tapetum and the inner layer of the sporangial wall. By division of adjoining cells the tapetal layer is extended completely around the sporogenous tissue (*fig.* 2), and by January, or earlier, the microspore mother-cells are formed and ready for their division in early spring. Chamberlain ('98) has reported a similar stage during winter in the microsporangia of Pinus, Cupressus, and Taxus. The cells of the whole sporophyll, with the exception of the tapetum and the sporogenous tissue, contain starch through the late fall and winter until renewed growth in spring alters its arrangement. In the middle of November the nuclei of the tapetal layer show a peculiar structure not found at other times. They have a very coarse and wide-meshed reticulum, upon which the chromatin is distributed in large granules of very unequal size. There is no nucleolus. The nuclei of the sporogenous tissue have several nucleoli and a thinner reticulum than at a later stage.

No trace of an indusium-like outgrowth from the sporophyll is present for the protection of the sporangia, such as occurs in Cupressus, Thuja, and species of Juniperus. During early stages of development the cells of the upper part of the sporophyll are completely filled with a peculiar homogeneous substance staining bluish with gentian, which, as its subsequent history shows, is either a form of starch or an intermediate product in the formation of starch. It is not stained blue by iodin. At the stage of *fig.* 1, this substance is being replaced by starch grains of the usual kind, and a direct relation in amount between the two is evident, the starch appearing in proportion as the amorphous substance disappears. The cells on the line of transformation contain both starch and amorphous substances in proportionately smaller quantities.

Before their division in the spring, the pollen mother-cells become filled with starch, while the grains in other parts of the

sporophyll are being rapidly corroded. The persistence of this starch in the mother-cells during division and its disappearance as the exine is formed in the pollen grain agrees with what is already known in cycads and conifers. The ripe pollen grain contains no starch, nor is any found in the pollen tube until it appears in the protoplasm of the central cell shortly before the formation of the sperm cells. The number of microsporangia on a sporophyll may be as many as nine, seven being a common number. The wall of the mature microsporangium consists of but two layers of cells on the exposed surface, and in this respect Taxodium differs from the Abieteae, Taxeae, Cycadales, and Ginkgo, and agrees with the Cupresseae and Gnetales. The cells of the outer layer of the wall have the sides and inner faces strengthened by bands of cellulose, while those of the inner layer are very much flattened and poor in contents. The cells in the tapetum have very dense contents and are shorter and thicker than those of the inner wall. They disorganize at about the time that the division occurs in the pollen grains.

The division of the pollen mother-cells took place this year (1901) in South Carolina on March 6th, and both divisions were found on the same day, even in the same cone, but the stages found in the same sporangium are not quite so different as Coulter and Chamberlain ('01) figure for *Pinus Laricio*. Changes of the nucleus leading up to the first division were not present in my material, but good preparations of all stages during and subsequent to the metaphase of the first division show that the phenomena are similar in all essential respects to those described in detail by Strasburger ('00) for Larix.

The chromosomes, as arranged on the nuclear plate, are short and thick (*fig. 3*). They stand at right angles to the axis of the spindle, the fibers being attached to the inner ends. The splitting begins at the point of attachment and in favorable cases the line may be seen between the two halves in the as yet unseparated outer limb. Very soon after the splitting is completed and the daughter chromosomes begin to move to the poles, the fibers are seen to be attached to the middle of the

bent chromosomes and the inner ends of the latter are composed of four arms, lying side by side, and generally of the same length (*figs. 4–6*). The second splitting has evidently occurred and the arrangement is now just as in Larix as described by Strasburger ('00). The chromosomes remain thick and short as they approach the poles, and their number can be easily determined to be either eleven or twelve. Eleven are shown in *fig. 6* in polar view, and at least this number could be distinctly made out in other cases. Sometimes there seem to be twelve, but on account of the crowding in such cases I have never been sure of this number. Twelve chromosomes have been found by Belajeff ('94) and Strasburger ('92) in the pollen mother-cells of *Larix europaea*, by Blackmann ('98) in pollen mother-cells and oosphere of *Pinus sylvestris*, by Juel ('00) in the megaspore mother-cell of *Larix sibirica*, and by Chamberlain ('99) in the pollen mother-cells, endosperm, and jacket cells of *Pinis laricio*. It would thus seem from analogy that the number of chromosomes in the pollen mother-cells of Taxodium is also twelve rather than eleven.

The daughter nuclei (*fig. 7*) before the next division enter into a fairly well-developed resting stage. There is a distinct reticulum, if indeed a rather coarse one, and the chromatin is grouped in larger masses than in the reticulum of many resting cells, approaching more nearly the condition already described in the nuclei of the tapetal layer of the microsporangium in November. Strasburger ('00) describes such a condition in Larix, but tries to bring it in harmony with other cases by considering the network as spun out from the chromosomes. His distinction is not clear to me, and I think it must be acknowledged that the daughter nuclei of the first division may, at least in some cases, reach before their next division a relatively well advanced resting stage. From *fig. 7* it will be seen that the cell walls of the mother-cell have not disappeared at the time of tetrad-formation. In places the walls have begun to go to pieces, but in others remain entire and in close contact with their neighbors. No case was found where the final divisions were bilateral, as is sometimes the case in *Pinus Laricio* (Coulter and Chamberlain, '01).

The connecting fibers of the first spindle produce a distinct cell-plate, extending entirely across the cell before the nuclei have begun to divide a second time. On each side of the plate the starch grains are densely crowded. The chromosomes of the second division are single slightly curved rods, and are evidently of about the same size as the halves of the double chromosomes of the first division. The starch begins to disappear during the second division of the pollen mother-cell, and is completely used up during the formation of the exine of the pollen grains, which becomes quite evident in about three days after the last division. The nucleus of the fully formed but yet undivided pollen grain is evenly and coarsely granular and generally without a nucleolus (*fig. 8*).

About ten days after its formation the pollen grain divides. The spindle is very small and the chromosomes are proportionately longer than in the reducing division (*figs. 9* and *10*). This is the only division of the pollen grain, no sterile prothallial cell being formed, and it separates at once the generative cell from the tube cell. The former is flattened lens-shaped, concave toward the inside, and furnished with a distinct *Hautschicht* (*fig. 11*). This division occurs a few days before the pollen is shed, and it is in this condition that the ripe pollen reaches the nucellus (*fig. 12*). In the absence of any sterile prothallial cells, Taxodium agrees with the Cupresseae and Taxus, and differs from all other conifers and cycads. The number of sterile prothallial cells in the pollen grain of gymnosperms has been determined in the following cases: two in Ginkgo (Strasburger, '92), *Larix europaea* (Strasburger, '84), *Picea vulgaris* (Belajeff, '93), *Pinus silvestris* (Strasburger, '92), *Pinus Pumilio* (Coulter and Chamberlain, '01); one in Ceratozamia (Juranyi, '82; he occasionally found two in *C. longifolia*), Zamia (Webber, '97), Cycas (Ikeno, '99); none in Biota, Cupressus, Juniperus (Strasburger, '92), *Taxus baccata* and Juniperus (Belajeff, '93).

The great importance of correctly determining the number of divisions in the pollen grain has not been overlooked, and repeated sections, at all stages of the development of the pollen

from the mother-cell stage to the sprouting of the pollen tube, have been made from collections obtained in both 1900 and 1901, and I think it certain that there is but one division of the pollen cell in Taxodium.

THE POLLEN TUBE.

The first indication of sprouting is given by the swelling up of the generative cell into the tube cell, and by an increase in size of both nuclei (*fig. 13*). The exine is usually thrown off at an early stage, as shown in *fig. 14*. In this figure the nuclei of the pollen tube have not changed their position, the tube nucleus lying immediately above the generative cell. The pollen tube contains no starch, either now or during its course to the prothallium. As the tube advances, the tube nucleus moves from its position over the generative cell and passes slowly down toward the tip. Indications of branching are soon seen in the pollen tube (*figs. 17, 29, 22, 23*). In *fig. 16* the generative cell seems by its position to be bounded by an actual membrane, but no indication of a cellulose wall was obtained, and if one is present it is exceedingly thin and quickly dissolved. By comparing *figs. 15* and *16* it will be seen that the sprouting does not take place at any definite point in reference to the position of the generative cell.

The division of the generative cell does not occur until several weeks after the sprouting of the grain (*figs. 19–21*). The stalk nucleus soon loses its definite hold upon the protoplasm around it, although immediately after the division (*fig. 19*) it is still bounded by a distinct protoplasmic sheath. The central cell retains the characteristics which mark the generative cell before division. It is furnished with a distinct *Hautschicht* and has the shape of a double convex lens. It will be noticed that immediately after the division the stalk cell is larger than the central cell. Belajeff ('91, '93) describes these two cells as being of equal size in Taxus. In *Juniperus communis* he ('93) finds the outer cell to be smaller, while in *Picea vulgaris* the opposite is true. There is not much difference in size in *Pinus Laricio*, as figured by Coulter and Chamberlain ('91). It will thus be seen that Taxodium agrees with Juniperus in the relative size of the stalk and central cells immediately after their formation.

Belajeff ('91, '93) describes the stalk nucleus as passing the central cell as it wanders down the tube. Such a description could hardly be applied in Taxodium when the tube is at right angles to the axis of the spindle of the generative cell. The stalk nucleus is as near the tip of the tube as is the central cell, and they both wander down together until they reach the tube nucleus (*fig. 22*). It will be seen from *fig. 26* that the three nuclei of the pollen tube can easily be distinguished at this stage. The stalk nucleus is smaller than the tube nucleus, while the protoplasm of the central cell is distinct from that of the pollen tube. The stalk and tube nuclei now advance slightly ahead of the central cell (*fig. 23*), and this relative position is retained by the three nuclei throughout the subsequent history of the pollen tube. In *fig. 23* the stalk nucleus is still slightly smaller than the tube nucleus, but the structure of the two is the same. The male nucleus is very like the other two, its nucleolus being slightly smaller.

The pollen tube proceeds to the prothallium without interruption; the growth, however, is much slower in the upper part of the nucellus than in the lower. No particular tissue of the nucellus tip is set apart to nourish or guide the pollen tube. All of its cells contain more or less starch, but there is no grouping of starch in definite areas. The pollen tube may reach the megaspore before the formation of a cellular prothallium (*fig. 25*). So early an approach of the pollen tube to the sprouting megaspore has not been described in any other case, so far as I am aware. Jäger ('99) gives one figure of *Taxus baccata* showing a pollen tube almost in contact with a young prothallium, and I have found that in *Taxus baccata canadensis* the pollen tube may reach the level of the megaspore before the latter has divided even once. One case was found in this plant where the pollen tube has grown against and badly compressed the megaspore before the latter had advanced far beyond the sixteen-cell stage. It was so completely crushed that the stage could not be exactly determined.

Fig. 26 gives the structure of the contents of the pollen-tube at a slightly later stage than *fig. 25*. The two free nuclei are

now exactly similar and lie side by side immediately beneath the central cell. The latter has increased greatly in size, as has also its nucleus, and the protoplasm is seen to possess a radiate structure. We find in the nucleus of the central cell a distinct peripheral network, and a nucleolus, irregular in outline and evidently of a compound nature. This kind of nucleolus, which we here meet for the first time, will be found to occur also in the nucleus of the central cell of the archegonium. In one case the central cell was at the tip of the pollen tube, with the two free nuclei behind it. One of the latter was pressed so closely to the protoplasm of the central cell as to indent it slightly. Such an abnormal relation between the generative and free nuclei has been noted in *Pinus Laricio* by Coulter ('97).

The further changes in the central cell before its final division into the sperm cells are so remarkable and have been so neglected in other conifers studied that I shall go into them with some detail. *Fig. 27* represents the central cell after it has reached its full size. It is no longer spherical, but has become elliptical in section, the long axis being perpendicular to the axis of the tube. The protoplasm is seen to be radiating from the two poles of the long axis. At these poles are sometimes to be distinguished slightly more granular areas, from which the radiations seem to diverge. The protoplasm is very dense, finely granular and in thin sections can be seen to have a reticulate structure. The faint areas at the poles of the cells will at once suggest in position the blepharoplasts of Ginkgo, Zamia, and Cycas. In reality, the resemblance is entirely confined to their position. Dr. Webber has kindly shown me his preparations of blepharoplasts in Zamia, and their intense staining and large size make further comparison impossible.

The nucleus, which is about half of the diameter of the cell, has rather abundant reticulum and a fragmented nucleolus. In addition to these, there has appeared a finely granular material which does not seem to differ in any respect from the linin material in the egg nucleus to be described below. It is most abundant around the nucleolus, but extends to all parts of the nucleus. In *fig. 27* one of the free nuclei is seen closely appressed

to the *Hautschicht* of the central cell; the other free nucleus does not appear in the section. This is about the latest stage at which these free nuclei retain their normal structure. They very soon begin to go to pieces, and the protoplasm of the pollen tube at the same time begins to disorganize. It becomes more homogeneous and retains more tenaciously the safranin stain. The nucleoli and chromatin of the free nuclei become more or less broken up and collected into masses of different size, a process which we shall see corresponds exactly to what occurs in the nuclei of the jacket cells of the archegonium shortly before its final division. Concomitantly with the disorganization of the nuclei and cytoplasm of the pollen tube, there becomes evident in the cytoplasm of the central cell a number of bodies staining a deep red in safranin. They resemble exactly the plastin granules that we have seen to appear at the disorganization of the free nuclei, and that they are actually transferred from the latter into the central cell seems possible. *Fig. 28* is a central cell after the appearance of these granules. They are arranged in a circular manner at some distance from the nucleus, and it may be that this distribution is connected in some way with the concentric arrangement of the fibers. At the time of the appearance of the plastin granules in the protoplasm of the central cell, there seems to be a distinct connection at the base of the cell between its protoplasm and that of the disorganizing material beneath it. Hirase ('95) describes large bodies lying in the protoplasm of the central cell of Ginkgo between the nucleus and the blepharoplasts. Webber ('97) confirms this and says that in addition to the two large bodies smaller masses of similar material were observed in other localities of the cell. He speaks of them as extra-nuclear nuclein. It is easy to compare these bodies with those of Taxodium. They stain deeply with safranin in both cases, the principal difference being that in Ginkgo they are generally fused into two large masses which occupy a definite position in the cell.

The disorganized mass of nuclei and protoplasm at the tip of the tube never completely disappears before fertilization, and it may appear in the tip of the archegonium above the protoplasm

of the egg after fertilization. In *fig. 28* a number of scattered starch grains have made their appearance in the cytoplasm of the central cell. They retain their scattered position until finally arranged into the dense starch sheath immediately surrounding the nucleus of the sperm-cell.

Changes in the nucleus preparatory to the final division of the central cell had already begun at the stage represented in *fig. 27*. In *fig. 28* these changes have proceeded still further. The chromosomes are being prepared from the few thick conspicuous threads that are present. The linin granules have become organized into a reticulum, and this reticulum seems to be arranging itself as if in the preparatory stages of spindle formation.

No attempt was made to study in detail all stages of spindle formation in the division of the central cell, but in *fig. 29*, which shows an oblique view of the spindle, the formation of its fibers from the nuclear reticulum and the granular nature of the more peripheral fibers seems evident. This formation of the spindle from the fibers of the nucleus will be described in more detail in the division of the ventral canal cell. *Fig. 30* shows a late telophase in the division of the central cell, the connecting fibers still being evident. A clear area is noticed on each side of the cell plate, and this area later extends entirely around the sperm cells. The starch and plastin material are collected at the distal ends of the spindle, but after the separation of the two daughter cells the starch becomes arranged in a dense sheath immediately surrounding the nucleus (*fig. 31*). Just outside of this sheath the plastin granules form a more or less complete layer. Beyond them is found the clear area previously mentioned, and the surface is composed of a distinct membrane which sharply defines the sperm cells from the protoplasm of the tube. After the formation of the daughter nuclei, they again begin to fill with the linin granules or reticulum (the so-called metaplasmic substance of Strasburger) until, at the time of maturity, they are so dense as to make any distinction between the granular material and chromatin reticulum very difficult. A small nucleolus, however, can be dimly discerned (*fig. 31*).

The sperm cells are now mature, and fertilization almost immediately takes place. I think it probable that the sperm cells do not round themselves off completely until after the bursting of the pollen tube, for although sometimes separated as much as a quarter of their diameter from each other, I have never seen them while still in the tube without a flat face on the inner side. This remarkably complex structure of the sperm cell distinguishes Taxodium from any phanerogam hitherto described, with the exception of Ginkgo and the cycads.

Recent work on the conifers, which in the structure of the male gametophyte approach Taxodium, gives very little detail as to the structure of the sperm cells. In Taxus Jäger ('99) mentions radial striae in the periphery of both the sperm cell and its smaller, functionless sister cell, but gives no further details of the protoplasmic structure. Arnoldi ('98) says that in Cephalotaxus the protoplasm of the sperm cells, which are here of equal size, is densest around the nucleus. In his work on Sequoia ('99) he gives no details of the protoplasmic structure of the sperm cells, but says they resemble those of the Cupresseae. Blackman ('98) makes some interesting observations on the sperm cells of *Pinus silvestris*. He says: "It cannot be doubted that cytoplasm also passes over into the oosphere, for each generative nucleus in the pollen tube is clearly surrounded by its own layer of cytoplasm, as can be observed in the stage when the tube is already in contact with the oosphere." Also, "it may here be noticed that small bodies staining deeply with fuchsin S may be observed in the generative cell protoplasm." These, he says, resemble leucoplasts. "If leucoplasts are really present in the cytoplasm belonging to the generative cells, the general view that the male cell brings over no plastids to the egg appears to be directly contradicted." This is the only mention I have seen made of a distinctive protoplasm belonging to the male cells in any of the Abieteae.

Neither Belajeff ('91, '93) nor Strasburger ('79, '84) describe the structure of the sperm cells of the Cupresseae in detail, but in gross structure they seem almost identical with those of Taxodium. Strasburger ('84) says that the pollen tube of Juni-

perus contains very little starch at time of fertilization, and thinks it therefore the more remarkable that the fusion nucleus should be surrounded by so much starch. From comparison with Taxodium, however, it seems probable that starch is also present in the sperm cells of the Cupresseae and has heretofore been overlooked. We shall see that a comparison of the processes of fertilization in the two cases strengthens this view.

The tip of the pollen tube has not been found to possess a distinct pit, such as is described by Goroschankin ('83), Dixon ('94), and Blackman ('98) in other conifers. The whole tip of the tube is furnished with a thinner cell wall than is found above, and if any pit occurs it is rendered less conspicuous by the thinness of the adjoining wall.

THE OVULATE CONE.

In October of the year preceding the ripening of the seed, the ovulate cones of Taxodium appear as very inconspicuous axillary buds on shoots of the same year. They usually occupy positions near the tip of the branch, and vary greatly in the number formed. It has usually been said that the ovulate cones are borne two or three together at the tip of branches which have also produced, further down, branches of the staminate inflorescence. While this is sometimes the case, and frequently so in trees examined in Baltimore, in its more natural habitat the ovulate cones are situated on branches of their own, and occur in much greater numbers than described. As many as fifteen or twenty mature cones have been found closely crowded on a fertile branch, and while this is the exception, as many as eight or ten are frequently grouped on vigorous trees. The ovulate cones replace the dehiscent short branches. The latter do not appear in the axils of all the scale leaves during the first year, but in only about one-third of them. The next year they are found in axils of leaves which were not occupied the previous year, but in following years they come from supernumerary axillary buds, and in the more slowly growing parts of the tree may appear year after year in the axil of the same scale leaf.

Fig. 32 shows a megasporophyll collected October 3, 1899.

In the axil of the sporophyll two swellings are present, the section passing longitudinally through one of them. These are the rudiments of the ovules which by January 4 (*fig. 33*) have begun to show the first indication of an integument. *Fig. 34* shows a sporophyll collected in Baltimore March 11. The ovule has increased in size and the nucellus and integument are of equal height. It seems that slow growth is continued all through the winter months whenever the condition of the weather will permit. A few weeks before the time of pollination, the placental outgrowth begins to appear as slight projections between and at the sides of the sporangia. By April 8 (*fig. 36*) the cushion has begun to show between ovule and scale, and by April 22 (*fig. 37*) it has reached a considerable size. The further development of the sporophyll is almost entirely confined to its basal part where cushion and scale are indistinguishable, and to the great extension of the cushion above and sidewise as a protective covering to the ovules. The tip above the cushion remains small and is soon much surpassed by the latter, which by fusing with the scale above soon comes to inclose completely the cavity in which the ovules lie. The outgrowths are not confined to the ovule-bearing scales, but are developed in almost as great degree in the axils of the adjoining scales below and at the tip. The number of fertile scales is usually about ten. They are bounded beneath and above by scales differing from them only in the absence of ovules. As the growth proceeds, the area of attachment of the ovules to the scale becomes much greater in extent, so that when the seed is mature almost the whole of its outer face is attached to the inner face of the scale.

This is not the place for a discussion of the homologies of the so-called placental cushion, and I shall confine myself to the expression of my belief that it is a new formation for the purpose of closing the opening between the scales for the protection of the ovules, and is not derived either from fused leaves or from a second integument of the ovule.

At the time of pollination the tip of the integument is composed of about three layers of cells, but immediately after pol-

lination the inner cells near the tip begin to grow in at certain points, and approaching the center almost completely close the micropylar cavity (*fig. 37*). There soon begins to appear in the tip of the integument an irregular ring of lignified cells with thick and pitted walls, which serves to strengthen this exposed end (*fig. 49*).

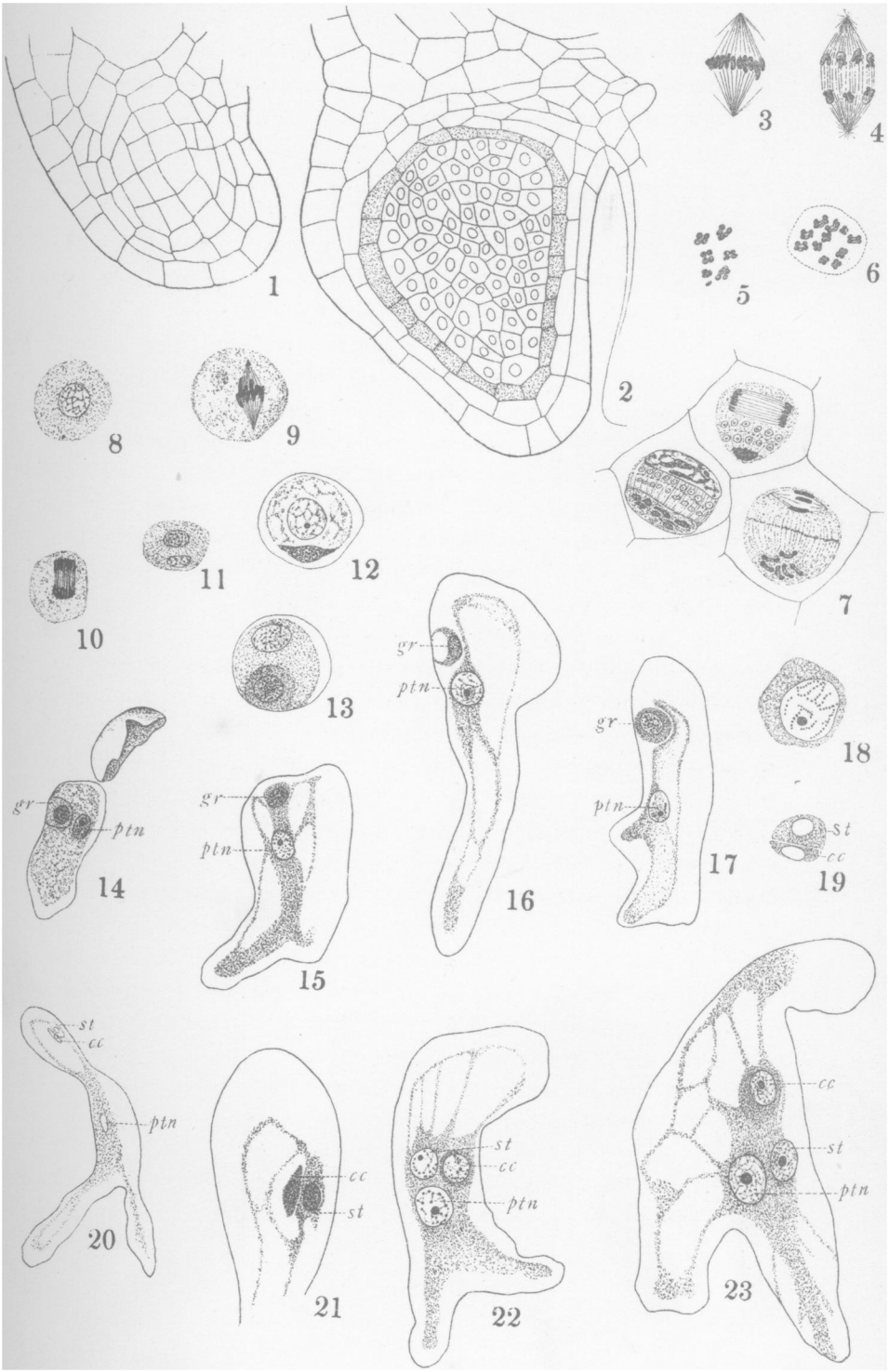
THE MEGASPORE.

The megaspore mother-cell cannot be distinguished from its neighbors until shortly before pollination. It is the basal or next to the basal cell of one of the distinct cell-rows which are evident in the center of the nucellus, and is situated at the same level as the point of insertion of the integument (*figs. 35, 37*). In this respect Taxodium agrees with the Cupresseae and differs markedly from Sequoia (Shaw, '96). At the time of pollination the megaspore mother-cell is slightly larger than the cells immediately surrounding it. Only a single megaspore mother-cell is present at this stage, but one case was found (*fig. 50*) in which the nucellus contained two young prothallia, one of which was larger than the other. Whether these were derived from two mother-cells or from the same mother-cell is a matter of conjecture. In this respect also Taxodium is seen to differ from Sequoia, in which Shaw ('96) and Arnoldi ('99[b]) have found it the rule for a number of prothallia to be developed. Hofmeister ('51) mentions the occasional presence of two prothallia in *Pinus silvestris* and *Taxus baccata* (confirmed since by Farmer ['92] for Pinus and by Jäger for Taxus), and I have found two in Podocarpus and *Taxus baccata canadensis*. With these exceptions the development of more than one prothallium has not been observed in the conifers.

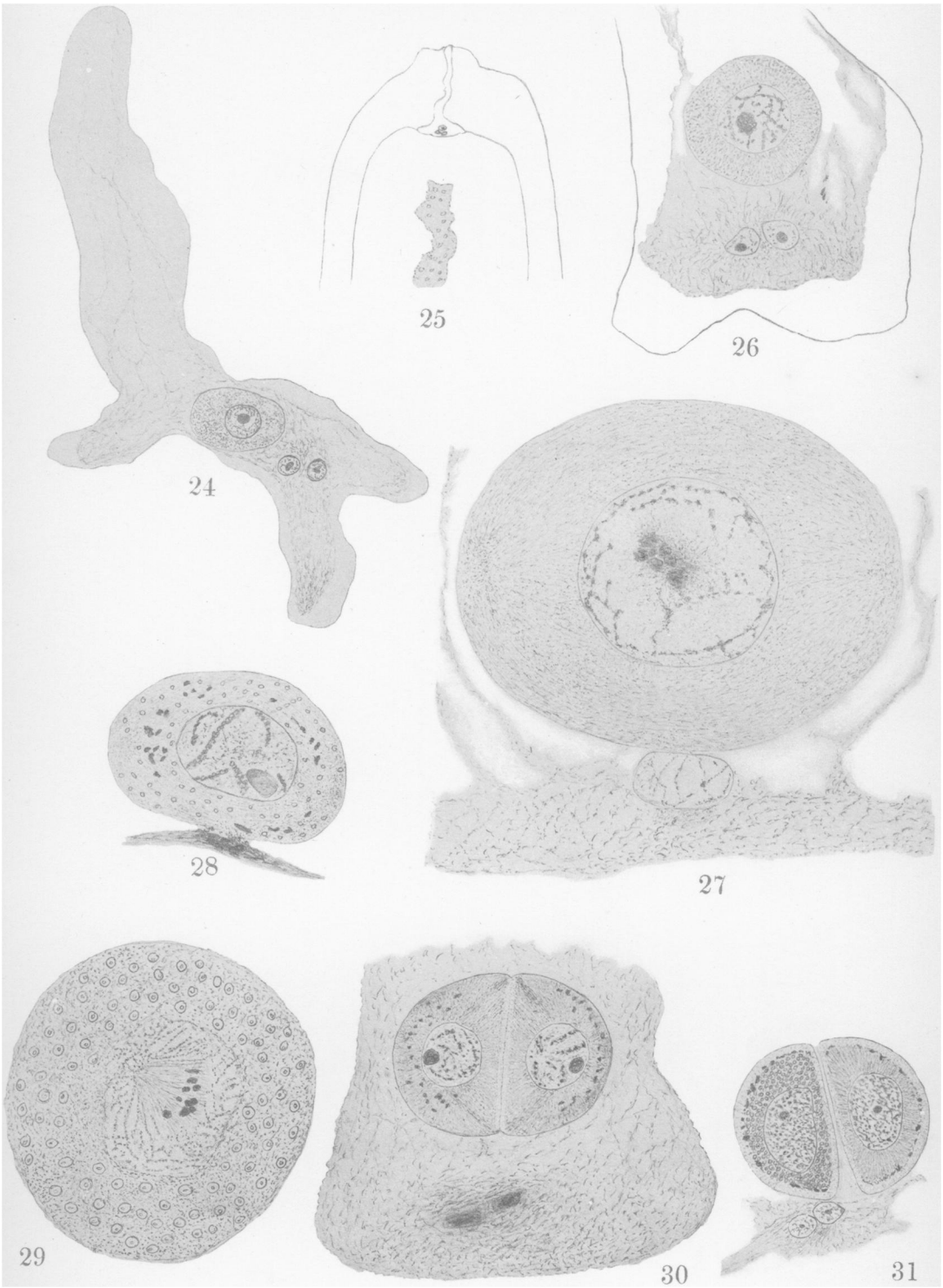
The mother-cell increases slowly in size after pollination, and in about ten days the first division occurs. *Fig. 39* is a mother-cell shortly before its first division. It is abundantly supplied with starch grains, as are also the adjoining cells for about one layer. It will be seen that the nucleus is in synapsis, the meshwork being in a contracted mass near the nucleolus. Such a synapsis is found by Juel ('00) at the same stage in *Larix sibirica*. He also mentions the presence of denser fibrous areas at

either end of the cell near the nucleus. These areas are frequently to be noticed in the megaspore of Taxodium, but I have not been able to establish any definite relation between them and the spindle-formation (*figs. 38–43*). A slightly younger stage is shown in *fig. 38*, where the nucleus is of the usual structure and has not approached the tip of the cell, in which position it is always to be found before the first division. Stages of the first division are shown in *figs. 40–42*. The chromosomes were not counted, but are evidently not far from twelve in number. This division cuts off a large lower cell and a much smaller upper cell. The lower cell immediately prepares to divide again. The second spindle is shown in *fig. 43*, and in *fig. 44* the division is complete. The starch has begun to disappear during these divisions, but some is present until the conclusion of the second. Strasburger ('79) describes it as disappearing in *Larix europaea* before the second division; the same is true in *L. sibirica* (Juel, '01) and *Pinus Pumilio* (Coulter and Chamberlain, '01).

The upper of the two cells formed at the first division does not divide again, and its nucleus never reaches the resting stage, or indeed approaches it. *Fig. 42* shows the difference in the nuclei of the upper and lower cells of the first division. The lower is developing as usual, but the upper has formed no reticulum, and in fact never reaches a more highly organized stage. Its chromosomes remain fused and lumped, and soon present merely a disorganized, homogeneous appearance. This history of the upper nucleus is repeated in detail by that of the upper cell of the second division. There are thus formed in Taxodium only three cells from the division of the megaspore mother-cell, but as the lower divides twice, it is in every respect the equivalent of a pollen grain, as much so as if the upper cell of the first division had divided, as is the case according to Juel ('00) in *Abies sibirica*. Strasburger ('79) gives three, or seldom more, as the number of potential megaspores derived from the mother-cell in Taxus. He also gives the same number in *Larix europaea*, but as Juel has found four in *L. sibirica* it is possible that Strasburger may have overlooked one in *L. europaea*. Coulter and



COKER on TAXODIUM.



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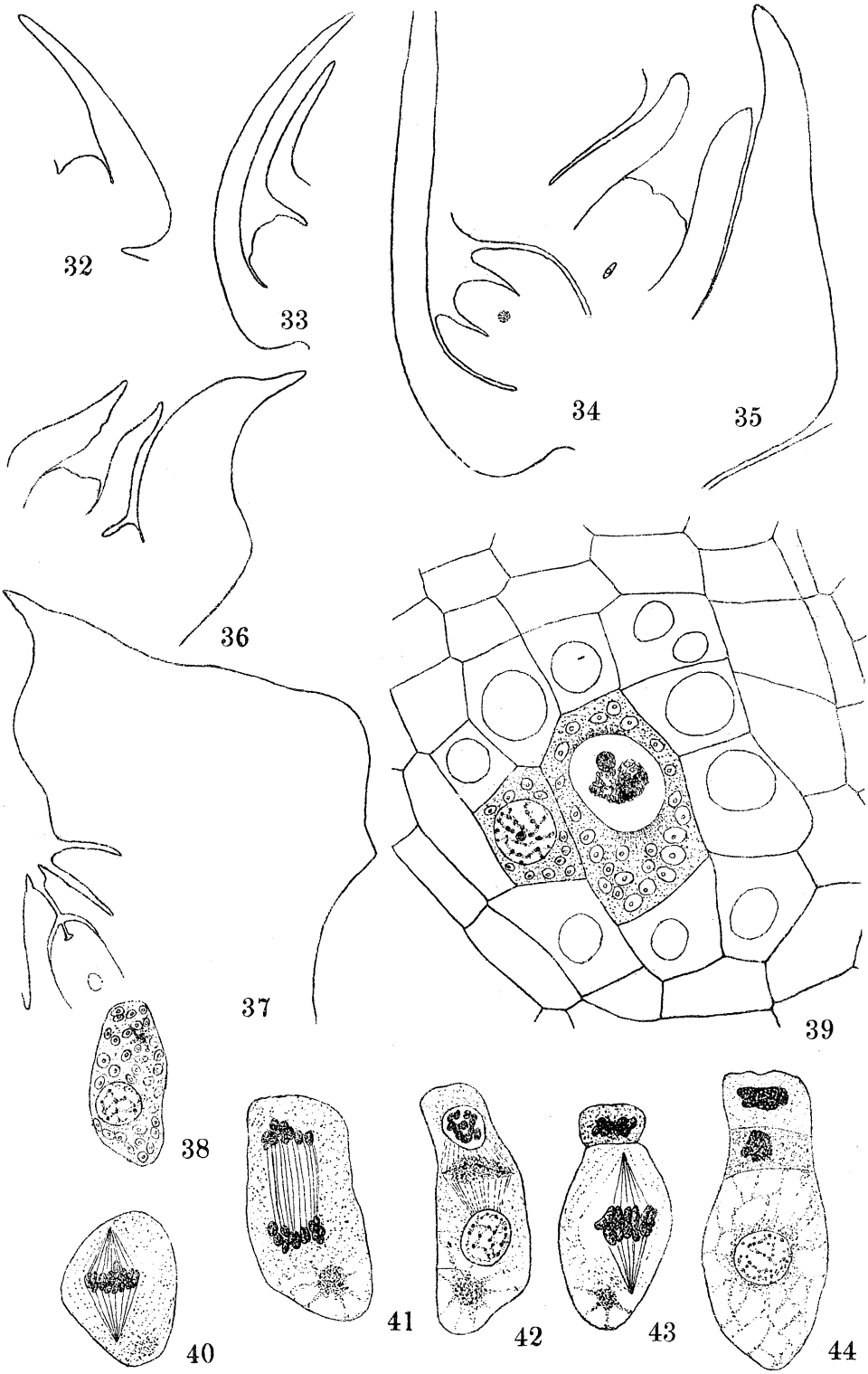

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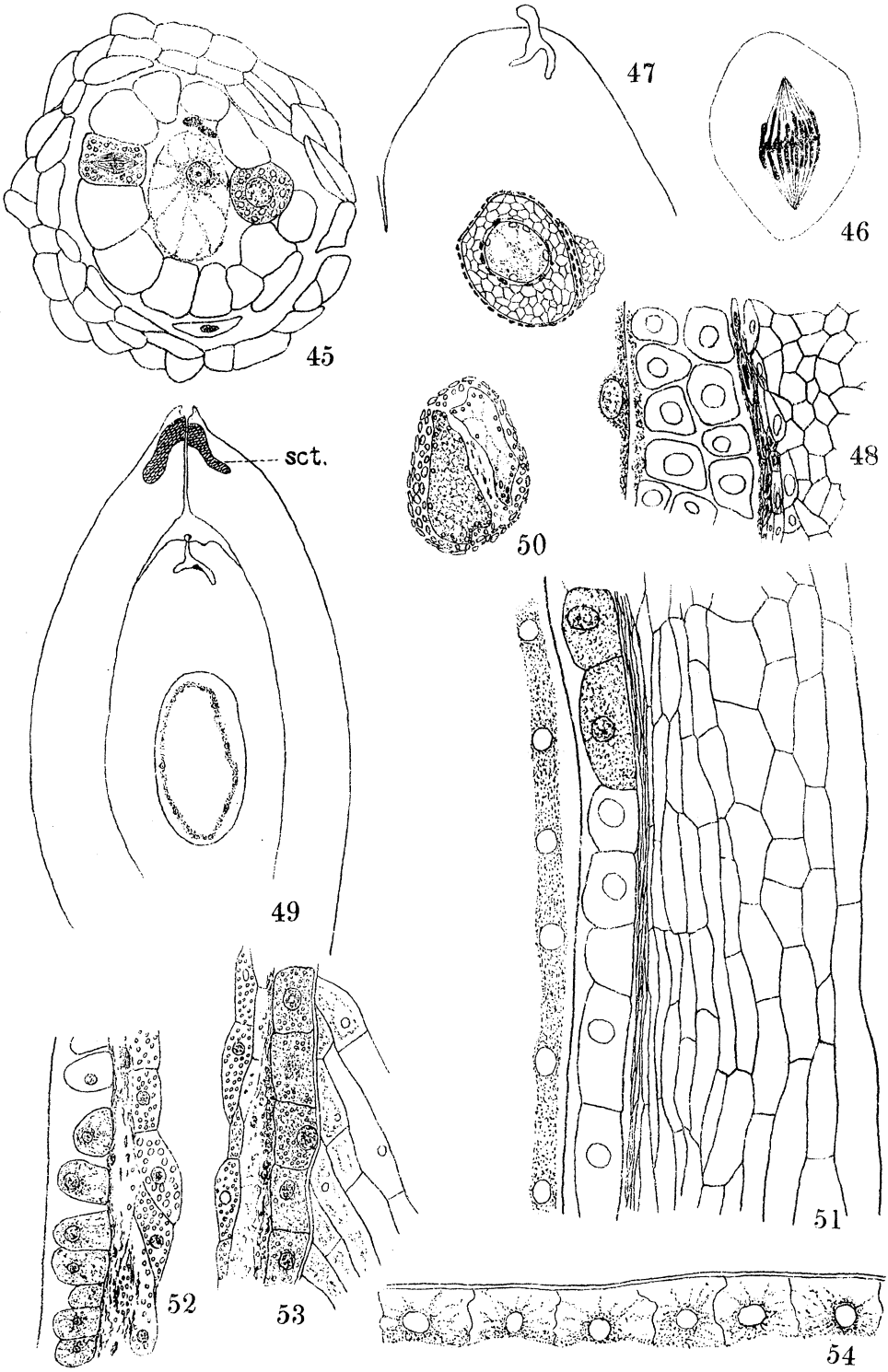

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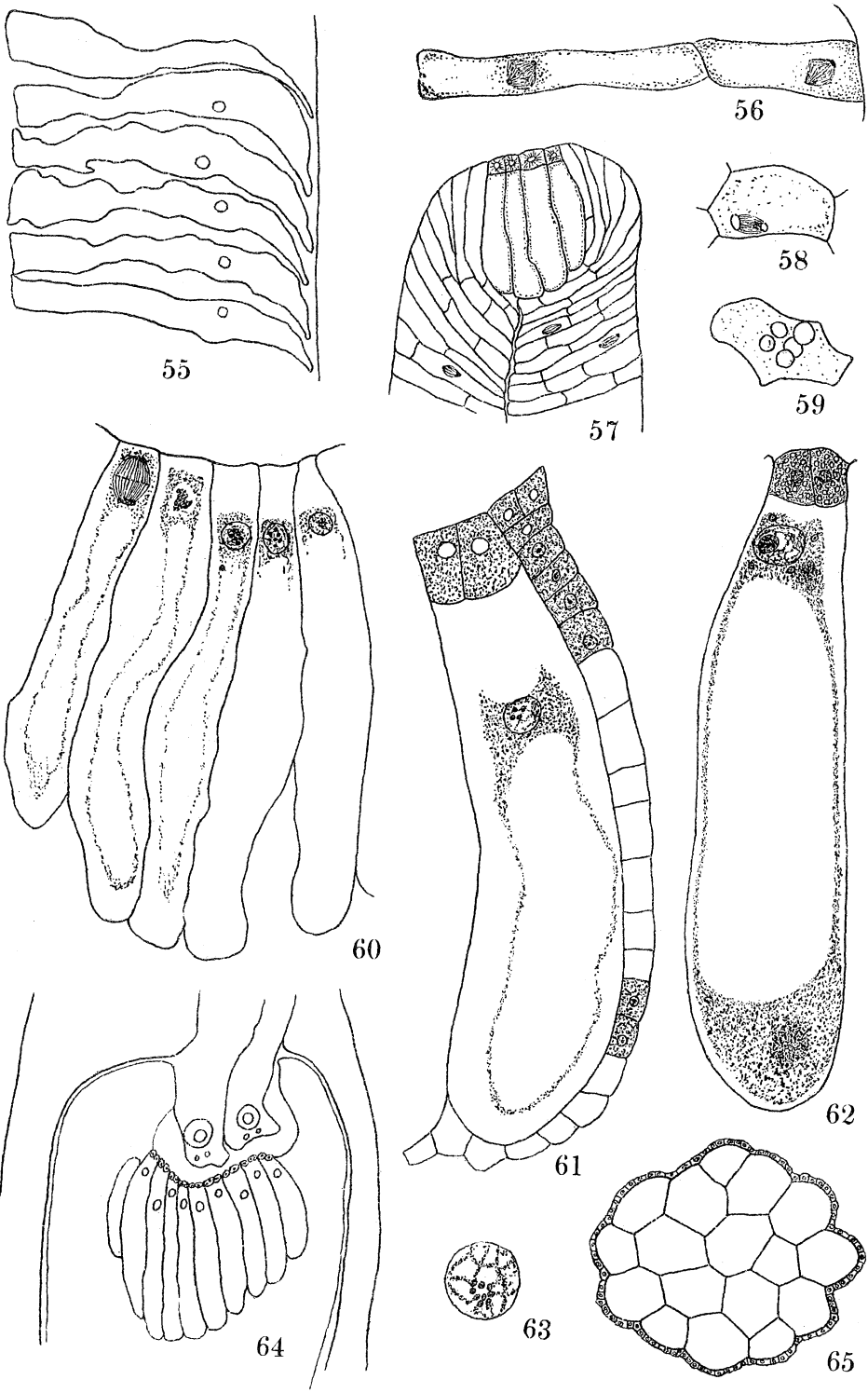

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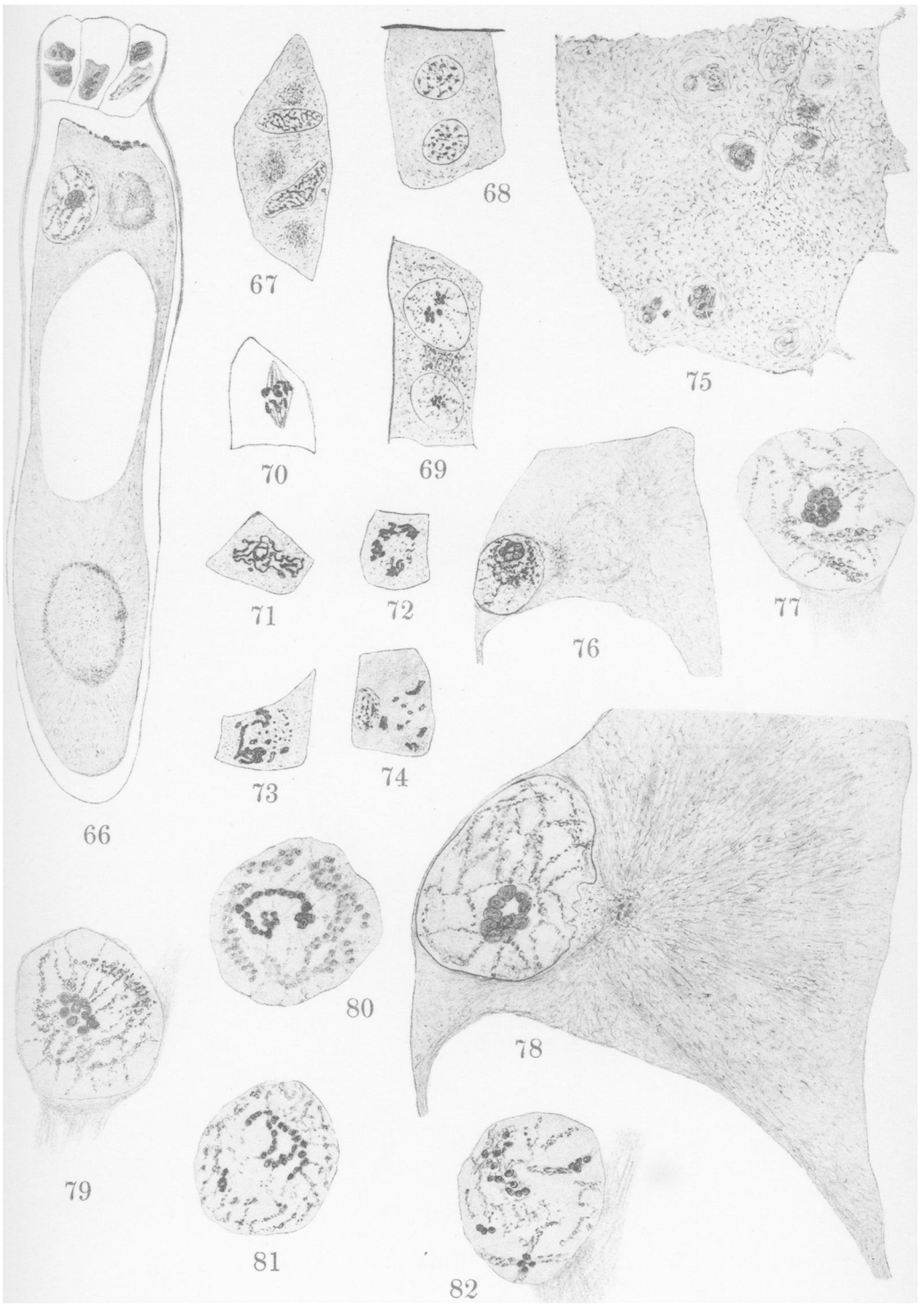

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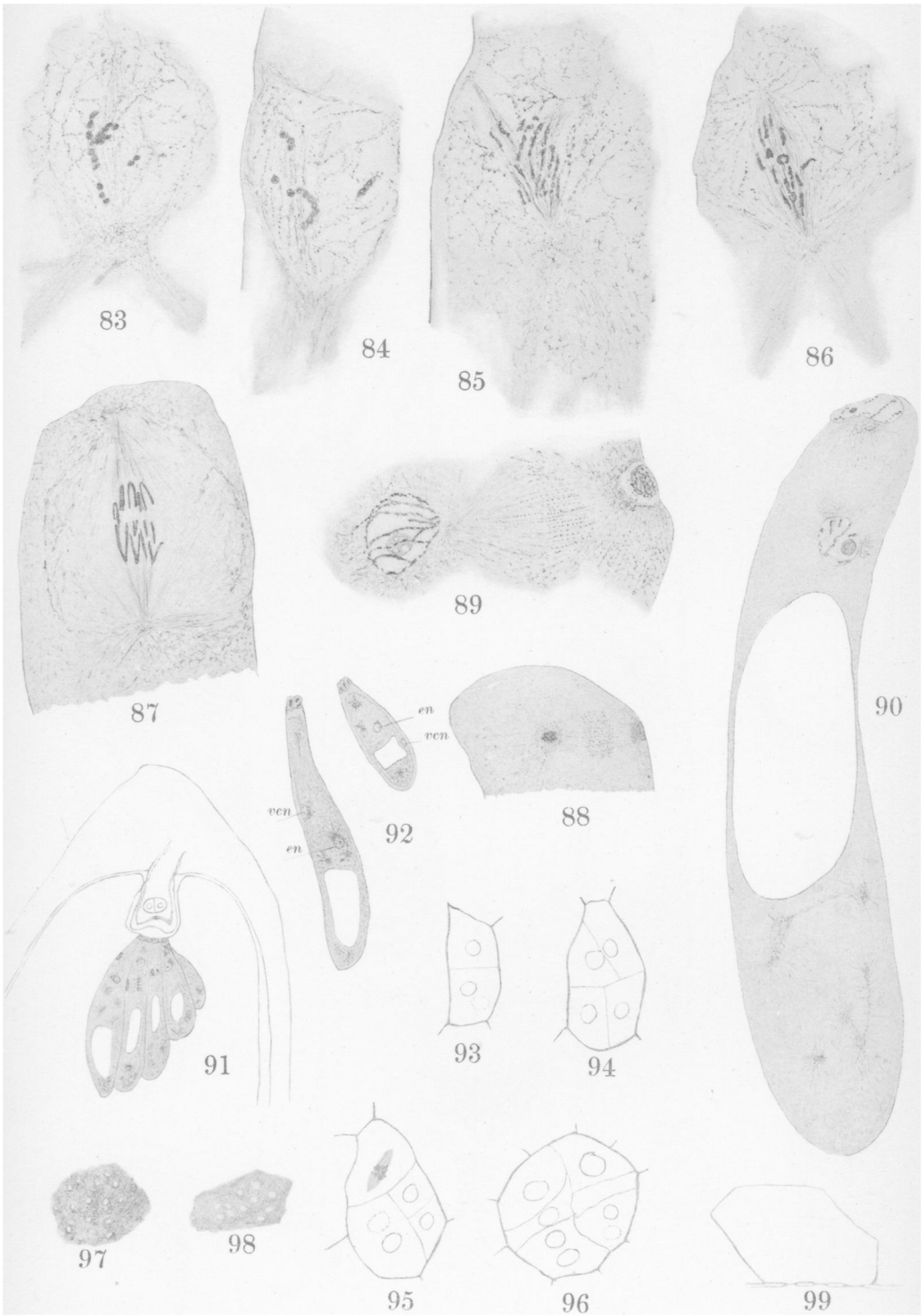

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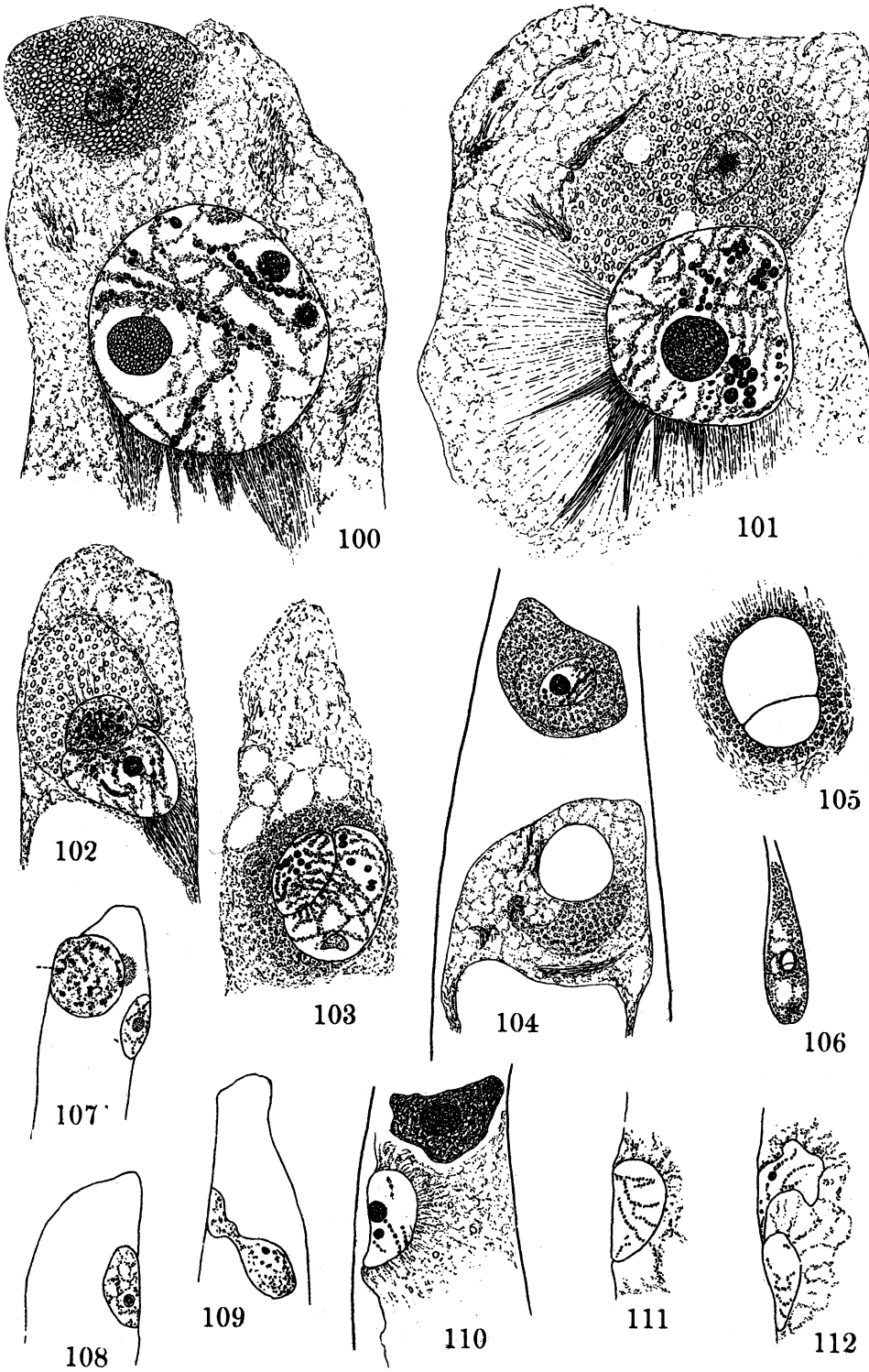

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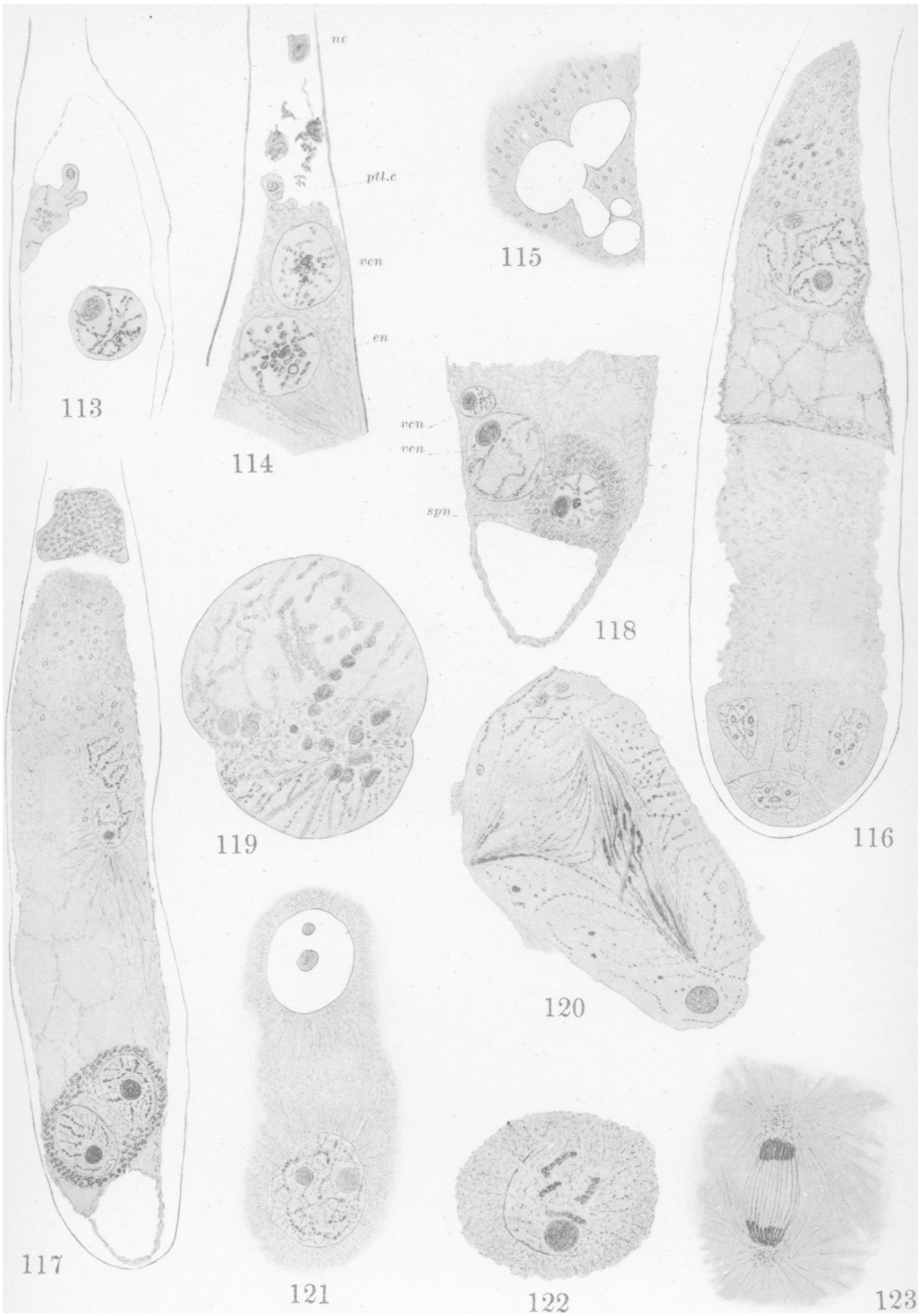

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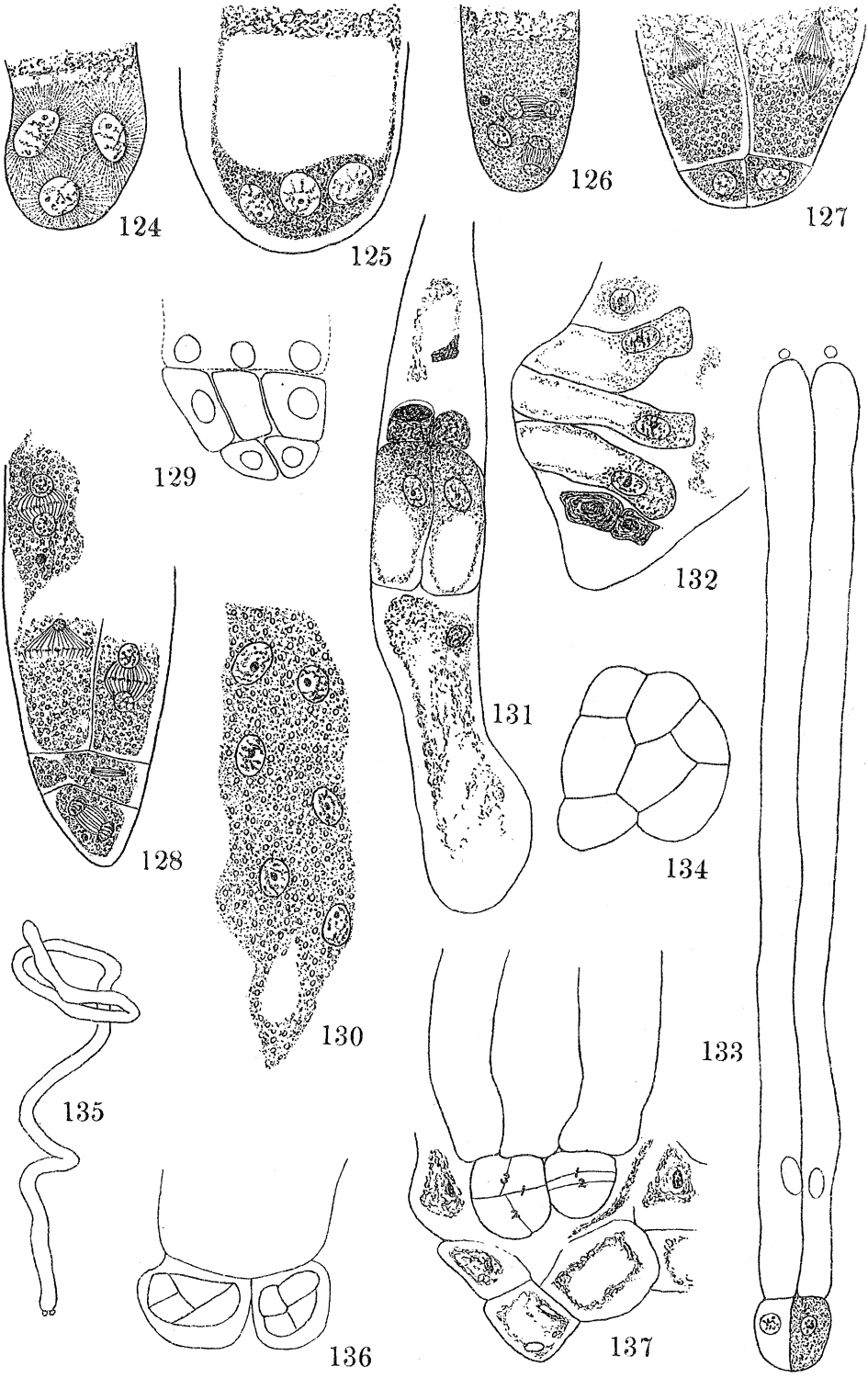

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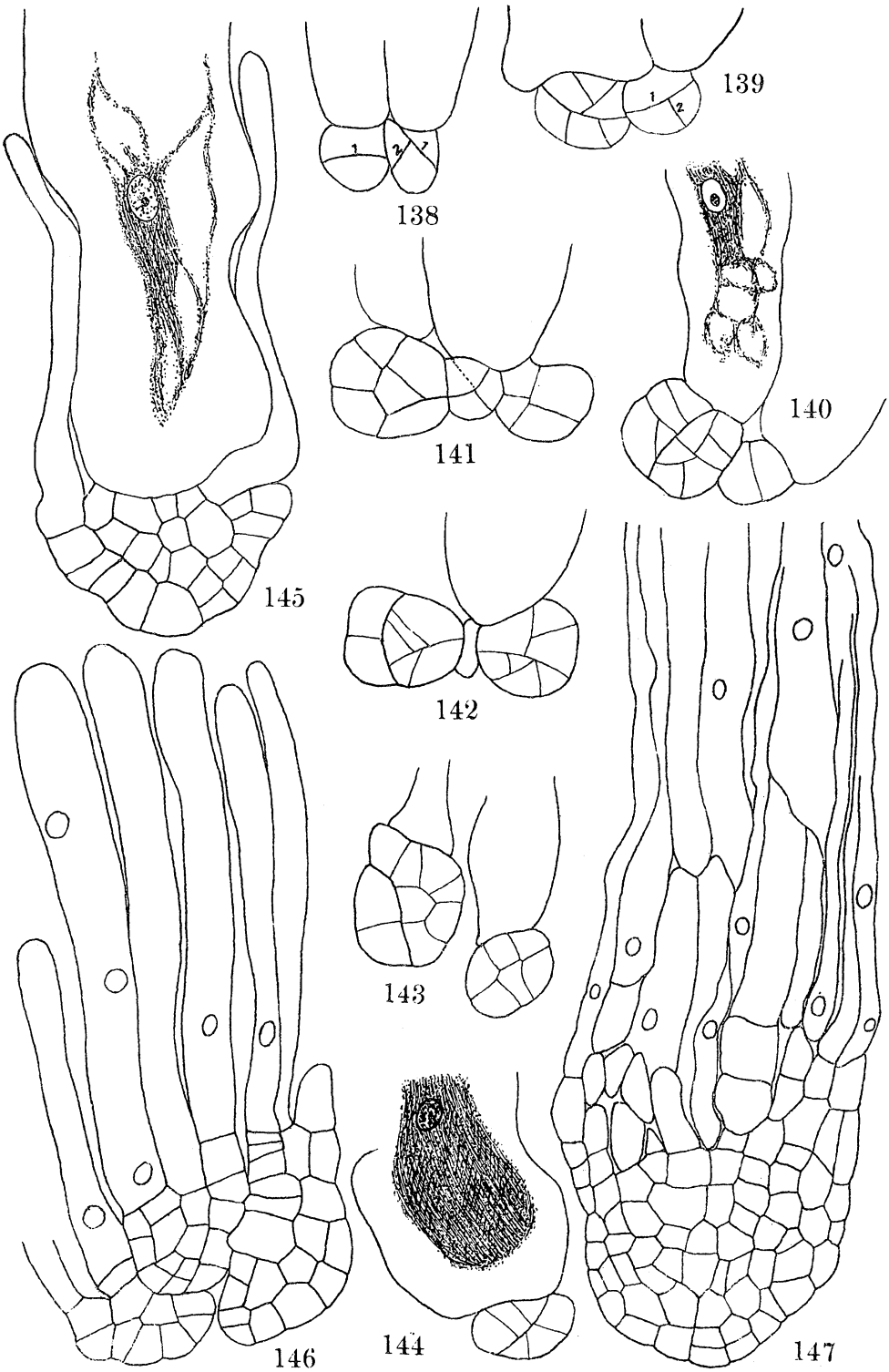

COKER on TAXODIUM.

Chamberlain ('01) also report four potential megaspores in *Pinus Laricio*, and Shaw ('96) has given the number as four in Sequoia. Almost nothing is known of the divisions of the megaspore mother-cell in the Cupresseae beyond Strasburger's remark that in Thuja the origin of the prothallium is essentially as in Taxus.

THE LARGE-CELLED TISSUE OR TAPETUM.

The cells immediately adjoining the megaspore mother-cell, as before stated, contain starch (*fig. 39*). After the megaspore is formed and begins to increase in size, the number of these dense, starch-containing, much enlarged cells is found to be greater (*fig. 45*). In *fig. 39* the transition between the starch-containing cells and the ordinary tissue around them is not very abrupt, but in *fig. 45* the boundary between the two has become distinctly marked. The larger cells are in close contact, their cell walls are intact, and their nuclei are large and apparently perfectly normal. Indeed, one of them is dividing mitotically, and they are not infrequently found dividing at this stage. *Fig. 46* shows such a cell in division. The spindle is placed centrally in the cell—not at one end, as in the division of the spore mother-cell—and in one case a cell-plate is being formed. The chromosomes were not counted, but the number is much more than twelve. The division seems to be an ordinary typical one. The cells immediately beyond the large-celled tissues are distinctly flattened and seem to be crushed by the cells within. On the lower side the nuclei of the nucellar tissue are frequently small, deeply stained in safranin, and apparently going to pieces. In one case at about this stage the nuclei in this position had entirely disappeared for a distance of several layers beyond the normal large-celled tissue. *Fig. 47* shows the large-celled tissue at a later stage. It has increased greatly in amount and the cells, which are now slightly separated from each other, are much more numerous. They retain the characters mentioned above for a younger stage.

The nucellar cells bordering on this tissue now show unmistakable signs of disorganization. They are completely crushed and broken up and are as strongly distinct from the large-celled

tissue as the latter is from the megaspore. *Fig. 48* is a more magnified section of the same spore and tissue. A small amount of granular material appears between the spore and the large-celled tissue, but the inner walls of the latter are apparently intact. A few cases were found, however, where the innermost of the large cells seemed to be partly broken up on the side next the spore. At the stage shown in *fig. 49* the existence of so definite a layer around the spore is doubtful. The cells adjoining the spore are still larger than others beyond them, but in all cases that I have found they are more or less separated from each other and approach in appearance the loosened "spongy tissue" which has been described in other cases. In later stages, however, there constantly occurs a single layer of very large rectangular cells lying next to the megaspore, while beyond those the ordinary nucellus-tissue is crushed and disorganized. *Fig. 51* shows such a layer around a large spore before the formation of the cellular prothallium. The large cells seem perfectly intact; walls are present on both sides and the nucleus is large, has an abundant reticulum, and is more like what would be expected in an actively secreting cell than in a rapidly disorganizing one. *Figs. 52* and *53* show the same layer at a later stage, just before the prothallium has reached its full size. In *fig. 52* the wall of the megaspore has shrunken somewhat and the large cells have become more elongated and slightly separated. The layer of large cells and two or three layers of ordinary cells beyond it are always furnished with starch during the growth of the prothallium; the starch is continually accumulating in the further-removed cells as the inner ones are being disorganized. The large-celled layer is also destroyed at the last moment and the mature prothallium is surrounded at its upper end with four or five layers of ordinary nucellar cells. In the Abieteae the young germinating megaspore is imbedded in a loose tissue which resembles somewhat the large-celled tissue just described in Taxodium. Its limits are so distinct that Hofmeister ('62) mistook it for endosperm. In comparing Strasburger's ('79) figures of such tissue in Pinus and Larix with my *figs. 45* and *47* in Taxodium, it will be seen

that there is a great difference in structure in the two cases. Moreover, this tissue in the Abieteae is always spoken of as disorganizing. Coulter and Chamberlain ('01) have an interesting paragraph on this subject. They say: "In our figure of a mother-cell of *Pinus Laricio* imbedded in nucellar tissue, it is apparent that it is surrounded by a rather definite zone of cells, two to four layers in depth, which give evidence of breaking down. After endosperm-formation is somewhat advanced, this interesting zone becomes differentiated into two distinct regions, an outer layer of tabular, almost empty, cells, and an inner region of polygonal cells with densely staining contents." I do not know of any case, however, where this layer is said to persist in later stages. Strasburger ('79) describes a zone of more or less disorganized cells around the germinating megaspore of Thuja. I have examined growing sacs of Pinus, Larix, Thuja, Podocarpus, and Taxus in reference to this point. At the base of the prothallium in Podocarpus and around the very young germinating megaspore of Thuja there are cells which approach, in appearance, those found in Taxodium. In fact, there are frequently present around growing prothallia a number of swollen free cells, which might be compared to such a tissue as I have described in Taxodium, but in most cases these cells are not in close contact with one another and their development can be gradually traced from the ordinary cells of the nucellus. But I have not studied any of these plants carefully enough to deny the persistence in them of this tissue, and further observation is necessary to decide the matter.

It is difficult to understand the constant occurrence of a definite layer of large, distinctive, undisorganized cells around the growing prothallium in Taxodium, unless we ascribe to them an active part in the nourishment of the young gametophyte, and this I believe to be their real function. If this interpretation is the correct one, the tissue in question may be considered as a tapetum, which, instead of disorganizing at the maturity of the spore, as is usually the case, has continued its growth to keep pace with the developing prothallium which it continues to nourish until mature. If we consider the archesporium as

reduced to a single cell (the megaspore mother-cell), the tissue immediately around this cell must be considered as a tapetum, and we have seen that it is probably by the division of this tissue that the nourishing layer is formed. This cannot as yet be positively asserted for the later stages, as all steps have not been followed. The only other interpretation that seems possible is that the cells immediately surrounding the megaspore represent an originally archesporial tissue which has given up its function of spore-production and taken up the new rôle of nourishing the young plant within. Of these two interpretations I consider the first as much the more likely.

It is well known that the tapetum in the megasporangium of Selaginella persists intact until some time after the sprouting of the spores, which in this case are shed only after a considerable growth has occurred. Should the spores not be shed at all, and the tapetum continue still further its growth and function, we would have a condition paralleling that found in Taxodium.

DEVELOPMENT OF THE PROTHALLIUM.

We have left the megaspore to follow the surrounding tissue through its subsequent stages. The growth of the germinating megaspore is extremely slow during the first month after its formation, having reached on April 29 only about the 32-celled stage (*fig.* 47). Growth now becomes more rapid, and on May 6 it has reached the stage shown in *fig.* 49. About the beginning of June growth has stopped in the upper part and the formation of cell walls begins. *Fig.* 25 shows a stage just before the beginning of cell-formation. The pollen tube has already reached the megaspore, which now contains an enormous vacuole surrounded by a protoplasmic layer containing nuclei. In this case the protoplasmic layer has collapsed.

The great size reached by the germinating megaspore before the formation of cellular tissue seems to distinguish Taxodium from other conifers thus far studied. The wall of the spore was found to be furnished with pits. *Figs.* 97 and 98 show those pits in surface view at a time shortly before the formation of a cellular prothallium. The manner of the formation of the

first cell walls in the young gametophyte is shown in *fig. 24*, and I can confirm Mlle. Sokolowa's observation, also recently repeated by Ikeno ('98) and Arnoldi ('00), that the inner sides of the first cells are open. Mlle. Sokolowa gives the name "alveoli" to the ingrowing tubular cells of the prothallium, but this term does not seem to have much to recommend it. It is repeated by Arnoldi ('00) in his work on Sequoia.

In view of the supposed relationship of Taxodium and Sequoia, it is of interest to compare the endosperm-formation in the two cases. Arnoldi has described in some detail a double process in Sequoia which he considers to have significance from the phylogenetic point of view. The prothallial region which is to bear the archegonia is formed in the usual way by ingrowing open tubes, which finally meet in the center. This area may be either in the center of the sac alone, or may extend entirely to the tip. In the latter case, however, Arnoldi considers the tip to be lacking. The tissue at the tip does not form in the usual way, but it is produced by free cell-formation, as in the endosperm of angiosperms. This free cell-formation begins in the tips before the tubes appear in the archegonial region. In Taxodium, on the contrary, cell-formation usually begins earlier at the tip, where archegonia are to appear, than at the base of the prothallium, and firm cell walls are frequently lacking in the lower parts long after they have been established in the upper. In fact, cases are not infrequent in which the lower part of the gametophyte is in an embryonic condition, even after fertilization has occurred in the archegonia. In some cases, however, firm cell walls seem to be formed almost simultaneously throughout the spore. In the upper part of the prothallium it is always easy to see that cell-formation has proceeded by the usual growing-in method.

Fig. 55 shows prothallial tubes from near the tip of the sac after their closure on the inner side. (Mlle. Sokolowa ['90] has shown that the closure occurs when the inner ends of the tubes meet in the center.) *Fig. 56* shows the first division of a prothallial tube and the preparation for the second. By these divisions there are formed rows of cells radiating from the

center outward. *Fig. 57* represents the whole tip of a prothallium at the same stage as in *fig. 56*. Mlle. Sokolowa describes the nucleus of the open prothallial tube as remaining at the inner end during its growth toward the center, but after the formation of the closing walls the nucleus again moves back to near the periphery of the cell. From *fig. 54* we see that the first statement is true in Taxodium, at least during the very young stages. Good preparations were not obtained by me in older prothallial tubes before the closure. From Mlle. Sokolowa's figure of Juniperus, it seems that the mother-cells of the archegonia behave in this respect just as the other cells of the prothallium, and this is probably true in Taxodium also.

The prothallial formation in the lower part of the spore does not appear to me to be different in essentials from its formation above. It is only in the late development of cell walls, and not in their peculiar origin, that the difference consists. The lower part, even after the formation of cell walls, continues to increase in size long after the upper part has ceased to grow. In the ripe seed the upper end of the nucellus is no larger than at the time of fertilization, while the lower part increases greatly in size, the whole prothallium acquiring the shape of a slightly bent club. After a certain number of cell walls are formed in the prothallial tubes, nuclear divisions occur without the formation of cell walls, and there arises a multinucleate condition (*figs. 58, 59*). These nuclear divisions are generally, at least, of the mitotic type. Jäger ('99) describes a fusion of nuclei in the prothallial cells of Taxus, but I have not found the number of nuclei appreciably smaller in Taxodium even after the formation of the embryo.

DEVELOPMENT OF THE ARCHEGONIA.

The archegonia of Taxodium are disposed exactly as in the Cupresseae. They form a compact group at the base of a common depression in the center of the tip of the prothallium. Among the many hundred prothallia sectioned only three or four were found in which any variation in this arrangement occurred. In these exceptional cases there were several smaller

groups of archegonia which were separated by a few layers of prothallial cells. They were always situated at the tip of the prothallium, but sometimes faced to one side.

In *fig.* 57 the initial cells of the archegonia are shown just before the cutting off of the neck cell. The nucleus is situated at the very tip of the cell and most of the protoplasm is collected around it. A very large vacuole occupies the greater part of the archegonium. In *fig.* 60 the neck cell is being cut off. The nuclei in the central initials are preparing to divide. Their nucleoli are fragmented, and although this nuclear division was not followed in detail, the indications are that it is essentially like that in which the ventral canal nucleus is cut off. *Fig.* 61 shows the neck divided once longitudinally. In *fig.* 62 the neck cells are densely filled with starch and the amount of protoplasm in the central cell has increased greatly, especially in the lower part. In the occurrence of a single large central vacuole in the archegonium Taxodium resembles the Cupresseae and differs from all other conifers so far studied. At this stage we first notice slightly denser areas in the protoplasm at each end. The upper lies very near the nucleus and is smaller than the lower, which occupies a central position in the accumulated basal protoplasm. The nucleus of the central cell at this stage is very like that of the prothallial cells around it. In *fig.* 68 there has already appeared around the archegonial group a distinct layer of sheath or jacket cells. At the basal end of the group the angles between the archegonia are filled by these jacket cells, which are at this point generally larger than on the sides. This jacket is a constant accompaniment of the archegonia in all gymnosperms with the exception of Welwitschia. Arnoldi ('00) reports that in Sequoia the layer is incomplete, only certain cells acquiring the distinctive characteristics of modified jacket cells. I have frequently found in Taxodium a cell within this sheath, which in its poverty of contents was easily to be distinguished from its neighbors, and resembles closely the ordinary prothallial cells around it. By far the larger number of the cells directly adjoining the archegonia, however, are modified in the usual way into the nourishing jacket cells. The nucleus of the central cell, at

the stage in *fig. 62*, is larger than the cells of adjoining tissue, but does not differ from them in structure. There is an abundant peripheral reticulum, staining blue throughout with gentian violet, and a nucleolus of compound structure, such as was found in the initial cell nucleus. In place of the single nucleolus there is frequently present a central group of quite distinct granules (*fig. 63*). All stages can be found between the distinct granules and the single compound structure formed by their fusion. From the nucleolus, or from the separate granules, linin threads extend which place the nucleolar matter in direct connection with the reticulum of the nucleus, and this I believe to be a constant character in nucleoli of chromatin material. That this nucleolus is largely composed of chromatin is shown by its subsequent behavior. As already mentioned, the nucleolar structure is the same in the prothallial cells and jacket cells, with the exception that in these the central or nucleolar collection of chromatin is quite inconstant in amount, the size of the nucleolus varying in proportion as the red-staining granules of the reticulum are more or less abundant.

The number of archegonia in a group varies greatly. *Fig. 64* shows a longitudinal section through a group of at least thirty-four archegonia, ten appearing in a single section. This is a larger number than has been found in any other gymnosperm, with the exception of Sequoia. The number is generally from ten to twenty, but in poorly developed prothallia there may be only a half dozen or less. *Fig. 65* is a cross section of a group of seventeen archegonia.

The neck cell very soon after its formation divides by a longitudinal wall into two cells of about equal size. This division is followed usually by another in each cell at right angles to the first, to form a tier of four cells (*fig. 94*). If the neck of the archegonium is crowded or flattened, the second division may occur in one or both of the two first-formed cells (*fig. 93*). As the archegonium reaches maturity, the nuclei of the neck cells generally divide again, and walls may or may not be formed succeeding this division. The walls when formed are very irregular in position. They are frequently somewhat inclined and

may or may not intersect the outer wall of the cell. In this way outer and inner cells are frequently formed (*fig. 66*), but two distinct tiers extending across the neck are never present. *Figs. 66, 93, 96* show the diversity that may occur in the neck. The cells are of unequal size and the number may vary from two to sixteen or even more. Very soon after their last division the neck cells begin to disorganize. They contain starch until the beginning of their disorganization, which takes place somewhat sooner here than in the jacket cells. *Fig. 66* shows an archegonium in which this disorganization is proceeding. There are present at the tip of the protoplasm of the central cell a number of bodies staining deep red with safranin, which may easily be mistaken for fragments of a disorganizing ventral canal nucleus. The last division, however, has not occurred in the central cell, and these bodies are probably the transferred remains of the broken-up nuclei of the neck cells. They are the first such bodies to appear in the archegonium, and in this stage are very conspicuous as a cap at its tip. This early transfer of nutrient material from the neck cells is probably explained by the advantage to be gained in having this transfer completed before fertilization has disturbed the relations between neck and central cell.

The jacket cells around the archegonia generally contain two nuclei each at this time (*figs. 67–69*), but this condition may be reached sooner. About the time that the ventral canal nucleus is cut off, the nuclei of the jacket cells begin to disorganize in the way already described in Cycas by Ikeno ('98) and in Ceratozamia by Arnoldi ('00). The network and the nucleoli resolve themselves into a number of deeply staining bodies, which by the disorganization of the nuclear walls come to lie free in the cytoplasm (*figs. 71–74*).

Immediately before fertilization there begin to appear in the cytoplasm of the egg the proteid vacuoles described in species of Abieteae. Such vacuoles are shown in *fig. 75* and have the same structure that has been described in other cases, but they are not very conspicuous or abundant in Taxodium. Arnoldi ('00) believes these vacuoles to originate in some cases from the

nuclei of the sheath cells of the archegonium, and thinks he has traced their entrance through protoplasmic connections in several species of Pinus and in *Abies sibirica*. From his work on Dammara and Cephalotaxus he is inclined to consider their origin as the same in these cases also. While I have not been able to establish the existence of protoplasmic connections between egg and sheath cells, the appearance in *fig. 83* strongly suggests that such connections exist, and the presence of pits in the wall of the archegonium would also imply their occurrence.

The two denser areas already noticed in the very young archegonium (*fig. 62*) have reached in *fig. 63* a size and condition retained until the initial changes which bring on the division into the central canal and egg nuclei. These areas are of dense fibrous material, and are by far the most striking features in the archegonium of Taxodium. They stain much more deeply with orange G than does the surrounding cytoplasm, and from them fibers radiate to the surface of the cell. It will be noticed that the denser part is at the periphery of the mass, but the inner part is also denser than the ordinary cytoplasm of the cell, and the whole is composed of a complex of granular fibers. The upper of these masses is the smaller and lies very near the nucleus. Fibers can be traced passing from the central mass around the nuclear wall, and they seem to be a continuation of the wall itself. That these bodies are of the same nature as the so-called kinoplasmic material, generally most conspicuous at the time of nuclear division, is evident. They show the same structure as the much less developed kinoplasmic areas already mentioned in the megaspore. Such bodies have not heretofore been described as occurring in such perfection in any case with which I am acquainted. Kinoplasmic areas have been mentioned near the nucleus at the time of its division in the archegonium of *Tsuga canadensis* by Murrill ('00), and Ikeno ('98) has figured such areas under the central cell nucleus of Cycas. Chamberlain ('99) gives one case where there are two such bodies near the egg nucleus in *Pinus Laricio*, and Blackman ('98) describes fibers of this nature radiating from the egg nucleus before fertilization. In all these cases, however, the fibrous material is not nearly so

conspicuous as in Taxodium, and in no case has a second kinoplasmic body been noticed in the lower part of the archegonium. Just before the central cell nucleus prepares to divide the kinoplasmic bodies undergo a marked change. They increase greatly in size, the central part becoming thinner, until finally the denser material, which has by this time become confined to a peripheral position, is broken up into more or less separate groups. In the upper end, one of these groups lying nearest the nucleus becomes most conspicuous, while the others become less and less distinct, their fibers finally arranging themselves into the immense aster which radiates from the inner side of the nucleus. Fibers extend around the nucleus from the center of the aster and merge insensibly into the nuclear wall (*fig. 78*). The lower kinoplasmic mass has also become broken up into separate parts, forming an incomplete ring, and during the activities in the upper part connected with the cutting off of the ventral canal nucleus these separated groups below become likewise extremely active and fill the whole base of the archegonium with conspicuous figures of various shapes, such as fans, asters, double asters, test-tube cleaners, etc. In fact the whole kinoplasmic content of the archegonium seems as if electrified. A few of these structures are shown in *figs. 90–92*.

Chamberlain ('99) figures such radiations in the egg of *Pinus Laricio* after the formation of the ventral canal nucleus, and considers them as parts of the enormously developed spindle of that division. In Taxodium, however, the kinoplasmic radiations in the lower part of the archegonium are in no way connected with the spindle above, although they are most active at the time of its formation. The function of these kinoplasmic masses is obscure, and I can suggest no explanation unless it be that the great length of the archegonia in Taxodium and the fact that it is principally at the end farthest away from the nucleus that they are exposed to the sheath cells may make it advantageous to have a more definite mechanism for the regulation of the entrance of the plastic material at this end.

[*To be concluded.*]